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TI A large-scale **gene-trap** screen for insertional mutations in developmentally regulated genes in mice.
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AB We have used a **gene-trap** vector and mouse embryonic stem (ES) cells to screen for insertional mutations in genes developmentally regulated at 8.5 days of embryogenesis (dpc). From 38,730 cell lines with vector insertions, 393 **clonal** integrations had disrupted active transcription units, as assayed by beta-galactosidase reporter gene expression. From these lines, 290 clones were recovered and injected into blastocysts to assay for reporter gene expression in 8.5-dpc chimeric mouse embryos. Of these, 279 clones provided a sufficient number of chimeric embryos for analysis. Thirty-six (13%) showed restricted patterns of reporter-gene expression, 88 (32%) showed widespread expression and 155 (55%) failed to show detectable levels of expression. Further analysis showed that approximately one-third of the clones that did not express detectable levels of the reporter gene at 8.5 dpc displayed reporter gene activity at 12.5 dpc. Thus, a large proportion of the genes that are expressed in ES cells are either temporally or spatially regulated during embryogenesis. These results indicate that **gene-trap** mutageneses in embryonic stem cells provide an effective approach for isolating mutations in a large number of developmentally regulated genes.